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# Evaluation of stream ecological integrity using litter decomposition and benthic invertebrates

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*This study supports the need for incorporating functional measures in evaluations of stream ecological integrity.*

**Abstract**

Biomonitoring programs to access the ecological integrity of freshwaters tend to rely exclusively on structural parameters. Here we evaluated stream ecological integrity using (a) benthic macroinvertebrate derived metrics and a biotic index as measures of structural integrity and (b) oak litter decomposition and associated fungal sporulation rates as measures of functional integrity. The study was done at four sites (S1, S2, S3 and S4) along a downstream increasing phosphorus and habitat degradation gradient in a small stream. The biotic index, invertebrate metrics, invertebrate and fungal communities' structure and sporulation rates discriminated upstream and downstream sites. Decomposition rates classified sites S4 and S2 as having a compromised ecosystem functioning. Although both functional and structural approaches gave the same results for the most impacted site (S4), they were complementary for moderately impacted sites (S2 and S3), and we therefore support the need for incorporating functional measures in evaluations of stream ecological integrity.


*Keywords:* Aquatic hyphomycetes; Functional integrity; Sporulation; Structural integrity

## 1. Introduction

High human densities in coastal zones with large urban and industrial settlements and intensive agriculture causes great pressure on surface water bodies and consequent deterioration of water quality (Ferreira et al., 2004; Martínez Mas et al., 2004) and changes in riparian vegetation (Urban et al., 2006; Wooster and DeBano, 2006). In an attempt to revert this situation the first step is to assess the ecological integrity of water bodies.

Presently, biomonitoring programs to assess the ecological integrity of freshwater ecosystems rely exclusively on structural aspects of the aquatic communities (Barbour et al., 1999; EU, 2000). Benthic macroinvertebrates have been extensively used in this way through the application of metrics (Maxted et al., 2002) and biotic indices (Ferreira et al., 2004; Alba-Tercedor et al., 2006). However, ecological integrity of an ecosystem results from both structural and functional components (Gessner and Chauvet, 2002). While structural integrity relates to the quantitative and qualitative composition of communities and their resources, functional integrity refers to the rates, patterns and relative importance of ecosystem level processes (Gessner and Chauvet, 2002). Therefore, both structure and function should be addressed in biomonitoring programs if ecological integrity of streams, and not only structural integrity, is to be assessed (Bunn and Davies, 2000; Gessner and Chauvet, 2002; Young et al., 2004).

Decomposition of submerged litter, a key ecosystem level process in small woodland streams, has been shown to have potential to be used as a functional tool to assess organic contamination (Pascoal et al., 2001, 2003; Gulis et al., 2006; Lecerf et al., 2006). Several studies reported a stimulation of

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litter mass loss rates with increasing dissolved nutrients, resulting from both cultural activities (Suberkropp and Chauvet, 1995; Niyogi et al., 2003; Gulis et al., 2006) and experimental enrichment (Elwood et al., 1981; Gulis and Suberkropp, 2003a; Ferreira et al., 2006). This stimulatory effect is mostly carried out through the stimulation of the activity of aquatic hyphomycetes (Gulis and Suberkropp, 2003a; Ferreira et al., 2006), which are the major microbial players in litter processing (Pascoal and Cássio, 2004). In particular, sporulation by aquatic hyphomycetes has been demonstrated to be one of the most sensitive fungal parameters to nutrient concentration in water (Ferreira et al., 2006; Ferreira and Graça, 2007; Gulis et al., 2006). However, although organic pollution is the most common anthropogenic stress to freshwaters (EPA, 2006), in lowland streams this perturbation appears generally associated with alterations in habitat quality due to channelization and removal of riparian vegetation (e.g. Urban et al., 2006). This co variation of factors can create confounding effects in the use of litter decomposition to assess ecosystem functional integrity in lowland streams (Niyogi et al., 2003; Hagen et al., 2006).

In this study, we performed a stream rapid assessment to evaluate stream ecological integrity using benthic macroinvertebrate derived metrics and an adaptation of the Iberian Biological Monitoring Working Party (IBMWP) biotic index as measures of structural integrity, and oak litter decomposition rates as a measure of functional integrity. To conform to stream rapid assessment characteristics, benthic samples were taken only once, and the decomposition of oak leaves was assessed three times along a 63-day period, at each site. Both structural and functional approaches used here meet 10 of the 12 ideal biomonitoring tool criteria (including all rationale criteria) defined by Bonada et al. (2006) and should therefore give an accurate picture of the ecosystem health status.

## 2. Materials and methods

### 2.1. Study sites

Botão stream is a third order lowland stream located in central Portugal in the Mondego river catchment. Four study sites were selected along a 2.9 km reach at Botão stream: two sites upstream the village of Botão (S1 and S2), one within the village (S3) and another downstream the village (S4) (Table 1, Fig. 1). Site locations were reflected into differences in water and habitat quality. At each sampling date, during winter 2006, Soluble Reactive Phosphorus (SRP) concentrations were always higher at sites S3 and S4 than at sites S1 and S2 (up to four times; Table 1). The SRP and $NO_3$ loads were also higher at the two downstream sites than at sites S1 and S2 (20–23 mg SRP/s at sites S3 and S4 vs. 4 mg SRP/s at sites S1 and S2; 4–5 g $NO_3$/s at sites S3 and S4 vs. 3 g $NO_3$/s at sites S1 and S2). Nutrient concentrations were already high at site S1 possibly due to the presence of villages and agricultural activities upstream the study site. Site S1 was surrounded by a riparian corridor mainly composed of native alder (*Alnus glutinosa* (L.) Gaert.), willow (*Salix* sp.) and poplar (*Populus* sp.) trees. Site S2 crossed a monoculture of the exotic *Eucalyptus globulus* Labill., although it also had alder and willow trees in the riparian vegetation. The substratum was very diverse at both sites. Site S3 was highly affected by channelization with a cement wall on the right margin, removed riparian vegetation and sewer drainage directly into the stream. The substratum was composed, almost exclusively, of sand where large macrophytes had evolved. Site S4 was surrounded by agricultural fields up to the stream margins and the original riparian vegetation was replaced by sparse stands of the exotic *Phragmites australis* (Cav.) Trin. ex Steudel; substratum was mainly sand.

Table 1
Geographic, physical and chemical characteristics (mean ± 1SD) of the four study sites in Botão stream

| | S1 | S2 | S3 | S4 |
|---|---|---|---|---|
| Latitude | 40°18′54″ | 40°18′46″ | 40°18′21″ | 40°18′2″ |
| Longitude | 8°23′3″ | 8°23′13″ | 8°23′50″ | 8°24′2″ |
| Altitude (m a.s.l.) | 91 | 82 | 69 | 62 |
| Distance to source (km) | 6.5 | 7.0 | 9.0 | 9.4 |
| Catchment area ($km^2$) | 19.5 | 22.1 | 24.1 | 24.4 |
| Slope (%) | 4.0 | 3.8 | 3.1 | 3.1 |
| Width (m)[b] | 3.3 ± 0.02 | 3.5 ± 1.1 | 3.3 ± 0.3 | 4.2 ± 0.7 |
| Depth (m)[c] | 0.23 ± 0.02 | 0.22 ± 0.03 | 0.25 ± 0.07 | 0.23 ± 0.04 |
| Current (m/s)[c] | 0.81 ± 0.25 | 1.07 ± 0.27 | 1.13 ± 0.34 | 0.91 ± 0.11 |
| Discharge ($m^3$/s)[b] | 0.61 ± 0.15 | 0.76 ± 0.11 | 0.88 ± 0.14 | 0.86 ± 0.19 |
| Temperature (°C)[b] | 8.8 ± 4.0 | 8.9 ± 3.9 | 8.3 ± 4.3 | 10.7 ± 4.0 |
| Conductivity (μS/cm)[b] | 172 ± 21 | 172 ± 20 | 188 ± 22 | 201 ± 25 |
| $O_2$ (%)[b] | 111.0 ± 5.7 | 122.0 ± 1.4 | 108.5 ± 0.7 | 114.0 ± 0.0 |
| pH[a] | 7.6 | 7.5 | 7.4 | 7.3 |
| Alkalinity (mg $CaCO_3$/L)[a] | 36 | 34 | 34 | 40 |
| SRP (μg/L)[b] | 5.85 ± 0.95 | 7.49 ± 4.73 | 16.23 ± 8.67 | 15.68 ± 6.62 |
| $NO_3$ (mg/L)[b] | 5.81 ± 1.59 | 5.77 ± 1.61 | 6.22 ± 1.94 | 7.03 ± 2.03 |

$NO_2$ and $NH_4$ were below the detection limits (<0.01 mg/L) at all sampling dates and sites.
[a] $n = 1$.
[b] $n = 3$.
[c] $n = 9$.

In spring 2007, the habitat quality of the four sites was evaluated by the Fluvial Habitat Index (IHF) and the Quality of the Riparian Corridor Index (QBR). The IHF evaluates the ability of the stream physical habitat to support a given fauna, and considers seven items related to substrate, current velocity and depth, shadow, presence of elements of heterogeneity and aquatic vegetation (Table 2; Jáimez-Cuéllar et al., 2004; Pardo et al., 2004). The final IHF score is the sum of the scores obtained for each item. The higher the habitat heterogeneity of a stream, i.e. the higher the frequency of riffles, the number of different substrate elements, the velocity/depth combinations, the number of elements of heterogeneity (leaves, branches, roots, inside the stream) and the diversity in primary producers, the higher the number of taxa it is able to support (Voelz and McArthur, 2000; Pardo et al., 2004). The IHF decreased in a downstream direction indicating degradation of habitat quality from site S1 to site S4 (Table 2).

The QBR evaluates the quality of the riparian corridor, and considers four items: degree of cover, structure and quality of the vegetation and degree of naturalism of the channel (Table 2; Jáimez-Cuéllar et al., 2004; Suárez et al., 2004). The final QBR score is the sum of the scores obtained for each item. The QBR classifies the riparian corridor quality into five classes: I (QBR $\geq$ 95), very good quality, riparian vegetation without alterations; II (90 > QBR > 75), good quality, riparian corridor lightly altered; III (70 > QBR > 55), moderate quality, beginning of important alteration; IV (50 > QBR > 30), low quality, important alteration; V (QBR $\leq$ 25), bad quality, extreme degradation. The higher the quality of the riparian corridor, the better it will perform its functions of controlling water temperature, providing shadow and litter for the aquatic heterotrophic food webs, providing structural elements (i.e. branches, dead trees), reducing the entry of inorganic nutrients from adjacent fields, which results in increased habitat quality and decreased eutrophication. Both downstream sites (S3 and S4) had a severely degradated riparian corridor, site S2 had a moderate quality riparian corridor and site S1 was considered to have a riparian corridor with no major alterations (Table 2). The information on water quality (nutrient concentrations), habitat quality (IHF) and riparian vegetation quality (QBR) led us to consider site S1 as the reference site to which all other sites would be compared to.

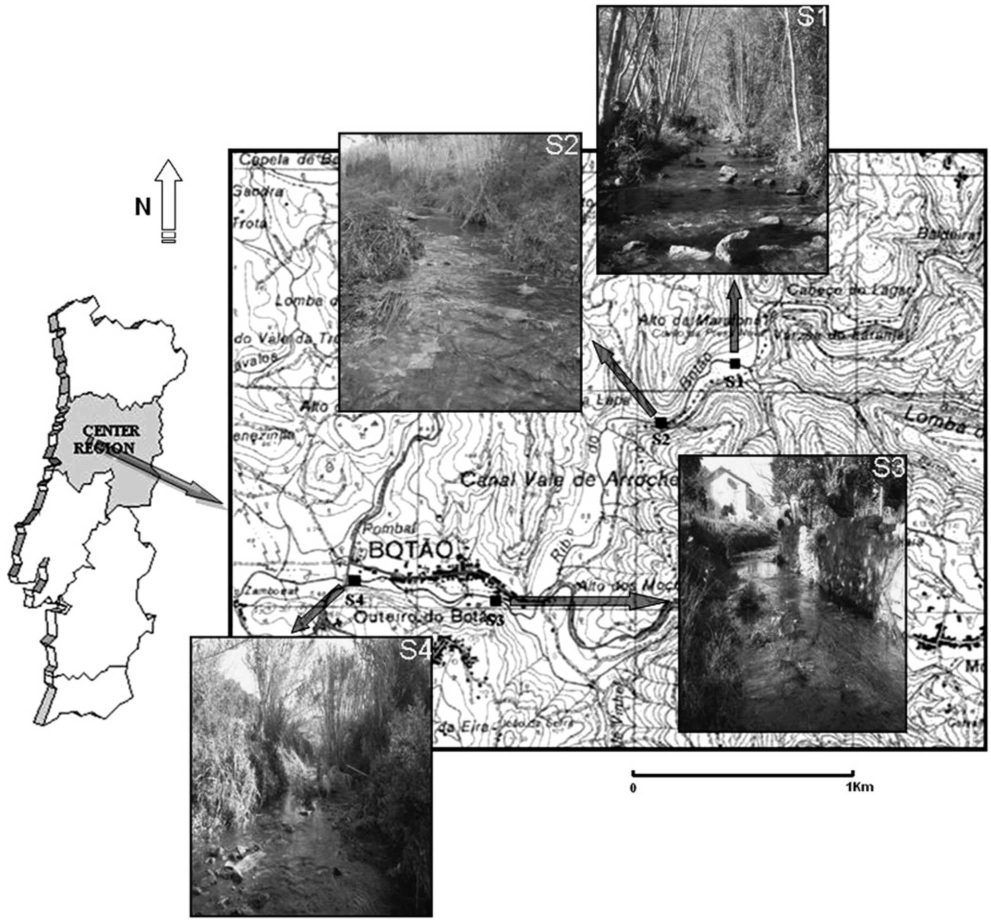


Fig. 1. Sites location in the Botão stream, in central Portugal. Photographs were taken on 1 February 2006 (d22 of the decomposition experiment).

Table 2
Evaluation of habitat quality at the four study sites in Botão stream

| | S1 | S2 | S3 | S4 |
|---|---|---|---|---|
| Fluvial Habitat Index (IHF) | | | | |
| Embeddedness in riffles/ sedimentation in pools | 20 | 20 | 15 | 5 |
| Frequency of riffles | 10 | 8 | 8 | 4 |
| Composition of the substrate | 14 | 17 | 11 | 14 |
| Velocity/depth combinations | 6 | 6 | 6 | 2 |
| Percentage of shadow in the stream | 10 | 5 | 3 | 3 |
| Elements of heterogeneity | 6 | 6 | 4 | 4 |
| Aquatic vegetation cover and diversity | 20 | 15 | 15 | 15 |
| IHF score [a] | 86 | 77 | 62 | 47 |
| Quality of the Riparian Corridor Index (QBR) | | | | |
| Degree of cover of the riparian corridor | 20 | 10 | 0 | 0 |
| Structure of the vegetation | 25 | 5 | 0 | 0 |
| Quality of vegetation cover | 25 | 25 | 20 | 10 |
| Degree of naturalism of the channel | 25 | 25 | 0 | 0 |
| QBR score[b] | 95 | 65 | 20 | 10 |

[a] IHF score is the sum of the scores of the seven items that compose it.
[b] QBR score is the sum of the scores of the four items that compose it. QBR $\leq 25$, extreme degradation; $70 > \mathrm{QBR} > 55$, beginning of important alteration; QBR $\geq 95$, riparian vegetation without alterations (as defined by Jáimez-Cuéllar et al., 2004).

### 2.2. Water characteristics

At each site and sampling date ca. 500 mL of water were filtered (Millipore APFF), stored in acid washed bottles, and transported to the laboratory in an ice chest. Water was analyzed for nitrate, nitrite and ammonia, using an ionic analyzer (Dionex DX-120). SRP concentration (determined by the ascorbic acid method; APHA, 1995) and alkalinity (measured by titration to an end pH of 4.5; APHA, 1995) were also determined. At the same occasion, stream width, depth, current velocity (VALEPORT 15277; which allowed to calculate discharge), dissolved oxygen (WTW OXI 92), pH (JENWAY 3310), conductivity and temperature (WTW LF 330) were measured.

### 2.3. Benthic macroinvertebrates

Benthic sampling took place on 1 February 2006. At each site samples were composed of six kicks (1 m each) taken in the most representative habitats with a hand net ($0.3 \times 0.3$ m opening and 0.5 mm mesh size). This was a conservative sampling when compared with that proposed by Alba-Tercedor (1996) and Jáimez-Cuéllar et al. (2004) where sampling should continue until no more families are identified after *in situ* sorting. Samples were preserved in 4% formalin until invertebrates were sorted. In the lab, each sample was washed through a sieve series (0.5–1.0–2.0 mm) to increase sorting efficiency. The macroinvertebrates were sorted and stored in 70% ethanol for further identification. Identification was made to the lowest possible taxonomical level (generally genus and species; Tachet et al., 2000). Invertebrates were classified into two groups: shredders and others (Tachet et al., 2000). The biomass of shredders was determined by measuring the total length of each individual under a stereoscopic microscope, and estimating biomass using body length–dry mass relationships (Meyer, 1989; Burgherr and Meyer, 1997;

Benke et al., 1999), except for *Tipula* sp. where the wet mass was determined and converted into dry mass by the relationship provided by Canhoto (1994).

Invertebrate data were used to calculate biotic metrics for each sampling site (EPA, 1999). The IBMWP biotic index (Alba-Tercedor, 1996; Jáimez-Cuéllar et al., 2004) was also calculated. For the application of the IBMWP index, we considered a family as present when more than one individual was counted. A score was then attributed to the family, and family scores were summed to determine the IBMWP value for each site. The IBMWP index classifies water quality into five classes: I (IBMWP $> 100$), very good water quality, water not contaminated or water quality not significantly altered; II ($100 \geq$ IBMWP $> 60$), good water quality, light contamination; III ($60 \geq$ IBMWP $> 35$), moderate water quality, some contamination; IV ($35 \geq$ IBMWP $> 15$), low water quality, high contamination; V (IBMWP $\leq 15$), bad water quality, very high contamination. The Iberian Average Score Per Taxon (IASPT) value for each site was calculated by dividing the IBMWP value of each site by the total number of IBMWP families present at each site (Alba-Tercedor, 1996).

### 2.4. Litter decomposition

Oak leaves (*Quercus robur* (L.)) were collected just after abscission, air-dried, weighed into 3-g groups and placed in coarse mesh (10 mm mesh) bags (15 × 15 cm). Oak leaves were chosen as a substrate for the litter decomposition experiment because in previous studies (Ferreira et al., 2006; Gulis et al., 2006) its decomposition was more responsive to nutrient concentration in water than that of the soft, nitrogen rich alder leaves. Incubation was done in coarse mesh bags (10 mm) to allow access to both microbes and invertebrates. A total of 48 bags were sealed and distributed at the sampling sites on 10 January 2006. In addition, six bags were taken to the stream and retrieved after 10 min of immersion to determine an initial dry mass to Ash-Free Dry Mass (AFDM) conversion factor taking into account handling losses. Sampling ($n = 4$) was done after 22, 41 and 63 days of immersion and bags were transported to the lab in individual zip lock bags in ice chests. Sixty-three days were enough to guarantee that mass loss of oak leaves would exceed 50% at the site where the decomposition rate was faster. In the laboratory, leaves were rinsed with distilled water to remove sediments and adherent macroinvertebrates, five leaf disks were cut out with a cork borer (12 mm diameter; see below) and the remaining mass was dried at 105 °C for 48 h, weighed, burned at 550 °C for 4 h, and reweighed to determine ash content and AFDM remaining.

### 2.5. Fungal sporulation

Leaf disks were incubated in 100 mL Erlenmeyer flasks with 25 mL of filtered stream water (glass fiber filter, Millipore APFF) on an orbital shaker (100 rpm) for 48 h at 15 °C to induce sporulation by aquatic hyphomycetes ($n = 3$). The conidia suspensions were fixed with 2 mL of 37% formalin for later counting and identification. When preparing slides, 100 µL of Triton X-100 (0.5%) solution was added to the suspension to ensure a uniform distribution of conidia, stirred and an aliquot of the suspension was filtered (Millipore SMWP, pore size 5 µm). Filters were stained with cotton blue in lactic acid (0.05%), and spores were identified and counted with a compound microscope at 200× (Bärlocher, 2005). Leaf disks were dried, weighed, ashed and reweighed as bulk material. Results were expressed as number of conidia/µg AFDM/day. Sporulation by aquatic hyphomycetes was the only microbial parameter determined as in previous experiments it was the most sensitive parameter to alterations in water quality (Ferreira and Graça, 2007; Gulis et al., 2006).

### 2.6. Data treatment

Ordinations of benthic macroinvertebrate samples considering all individuals and shredders alone were done by principal component analysis (PCA) as the length of gradient of axis 1 was <3 SD as determined by detrended correspondence analysis (DCA) (CANOCO v4.5; ter Braak and Smilauer, 1998). Axis 1 and 2 coordinates were related to water parameters and decomposition rates by linear regression. Decomposition rates ($k$/d) were calculated by fitting the mass loss data (ln transformed) into the negative exponential model ($M_t = M_i \times e^{-tk}$, where $M_t$ is the remaining mass at time $t$, $M_i$ is the initial mass, and $k$ is the decomposition rate constant). Comparison of slopes among sites was done by ANCOVA (sites as categorical variable and time as continuous variable) followed by Tukey's test (Zar, 1996). Relationships between decomposition rates and fungal sporulation, shredder numbers and shredder biomass were assessed by linear regression. Decomposition rates at sites S2–S4 were divided by the decomposition rate at site S1 ($k_{\mathrm{impacted}}$:$k_{\mathrm{reference}}$) as suggested by Gessner and Chauvet (2002) as a measure of ecosystem functional integrity.

Sporulation by aquatic hyphomycetes (log transformed), species richness and Pielou's equitability and Shannon's diversity indices (PRIMER v6) were compared among sites by two-way ANOVA (sites and time as categorical variables) followed by Tukey's test (Zar, 1996). Cumulative conidial production at a given sampling date was calculated by summing up values of daily production at each previous sampling date and linearly approximated values for each day between sampling dates. Comparisons of cumulative conidial production among sites were done by ANCOVA followed by Tukey's test. Percentage contribution of aquatic hyphomycete species to total conidial production was compared among sites by two-way ANOVA followed by Tukey's test (Zar, 1996). Aquatic hyphomycete communities associated with decomposing oak leaves were compared among sites, for each sampling date, by principal response curve (PRC) analysis (CANOCO v4.5). PRCs are suitable to be applied to field data collected through time in reference (here site S1) and treatment sites (here sites S2–S4). The analysis output is a graph with time on the $x$-axis and the deviations (compositional differences) of all treatments regarding the control on the $y$-axis (principal response); the control is assumed to be 0 at all instances (the $x$-axis itself). Species scores are also calculated and reflect the influence of each species over time on the overall community response as described by the PRCs. All analyses were performed with STATISTICA 6 software unless otherwise indicated.

## 3. Results

### 3.1. Benthic macroinvertebrates

The benthic macroinvertebrate sampling resulted in 2294 individuals distributed by 38 families, including 7 shredder families. Site S4 was the site with the lowest number of individuals and taxa (Table 3). Total number of taxa, number of EPT taxa, number of Trichoptera taxa, % of Trichoptera individuals and % intolerant organisms were higher at sites S1 and S2 than at sites S3 and S4, while % of Chironomidae individuals, % of Diptera individuals, % of Oligochaeta individuals and % tolerant organisms were higher at sites S3 and S4 than at sites S1 and S2 (Table 3). The IBMWP biotic index indicated that sites S1 and S2 had very good water quality (class I), site S3 had good water quality (class II) and site S4 had moderate water quality (class III). IASPT values were also higher at sites S1 and S2 than at sites S3 and S4 (Table 3), indicating higher sensitive families at upstream sites. Abundance, species richness and diversity of benthic shredders were also higher at the upstream sites than at sites S3 and S4 (Table 4). Biomass of shredders was higher at site S4 followed by sites S2, S3 and S1 (in decreasing order), which was related to the number of tipulids (the larger invertebrate; 71–550 mg individual mass), present at each site (Table 4).

The ordination of benthic macroinvertebrate communities (all individuals) by PCA (Fig. 2) discriminated sites S1 and S2 from sites S3 and S4; sites were distributed along axis 1, which explained 52% of variability, and were negatively related to the SRP concentration in water (linear regression,

Table 3
Selected benthic invertebrate parameters from four sites along a habitat degradation gradient in Botão stream

| | S1 | S2 | S3 | S4 |
|---|---|---|---|---|
| Total no. of individuals | 729 | 595 | 628 | 342 |
| Total no. of taxa | 34 | 38 | 25 | 21 |
| No. of EPT taxa[a] | 12 | 10 | 6 | 5 |
| No. of Trichoptera taxa | 6 | 4 | 1 | 2 |
| % Trichoptera individuals | 5.1 | 5.5 | 0.2 | 0.9 |
| % Diptera individuals | 35.9 | 46.1 | 61.6 | 50.9 |
| % Chironomidae individuals | 23.7 | 28.1 | 56.5 | 38.9 |
| % Oligochaeta individuals | 1.0 | 4.4 | 6.7 | 11.1 |
| % Intolerant individuals[b] | 26.6 | 24.4 | 17.4 | 3.8 |
| % Tolerant individuals[c] | 36.1 | 43.0 | 69.1 | 71.1 |
| No. of IBMWP families | 28 | 25 | 13 | 12 |
| IBMWP[d] | 168 | 147 | 61 | 49 |
| ASPT | 6.0 | 5.9 | 4.7 | 4.1 |

[a] EPT = Ephemeroptera + Plecoptera + Tricoptera.
[b] Intolerant individuals belong to families with scores of 7, 8 and 10 in the IBMWP index.
[c] Tolerant individuals belong to families with scores of 1, 2 and 3 in the IBMWP index.
[d] IBMWP value is the sum of the tolerance scores of families (score = 1–10, the higher it is the more sensitive the family is to organic pollution) represented by >1 individual. The IASPT value is the average score per taxon (=IBMWP/number of IBMWP families). IBMWP > 100, very good water quality; 100 ≥ IBMWP > 60, good water quality; 60 ≥ IBMWP > 35, moderate water quality (as defined by Alba-Tercedor, 1996; Jáimez-Cuéllar et al., 2004).

$p = 0.007$, $R^2 = 0.98$), and to the SRP and $NO_3$ loads (linear regression, $p = 0.031$ and 0.030, respectively, $R^2 = 0.94$ for both). Axis 2 explained 28% of variability but was not related with any measured parameter. The ordination (PCA) of the shredder communities resulted in the separation of sites S3 and S4 from the other two, which were also apart from each other (axis 1 explained 62% and axis 2 25% of variability; data not shown). No relationship was found between sites coordinates and water parameters or *k* values.

Table 4
Benthic shredders from four sites along a habitat degradation gradient in Botão stream

| | S1 | S2 | S3 | S4 |
|---|---|---|---|---|
| Leptophlebiidae | | | | |
| *Paraleptophlebia* sp. | 5 | 2 | | |
| Capniidae | | | | |
| *Capnia* sp. | | | 10 | 1 |
| *Capnioneura* sp. | 12 | 3 | 3 | |
| Nemouridae | | | | |
| *Nemoura* sp. | | 4 | | |
| Calamoceridae | | | | |
| *Calamocerus marsupus* | 2 | 4 | 1 | |
| Lepidostomatidae | | | | |
| *Lepidostoma hirtum* | 6 | | | |
| Limnephilidae | | | | |
| *Halesus* sp. | 1 | | | |
| *Limnephilus* sp. | 7 | | | |
| Tipulidae | | | | |
| *Tipula* sp. | | 4 | 1 | 9 |
| No. of shredder individuals | 33 | 17 | 15 | 10 |
| No. of shredder taxa | 6 | 5 | 4 | 2 |
| Total shredder dry mass (g) | 0.06 | 1.00 | 0.23 | 3.05 |
| % shredders | 2.9 | 2.4 | 1.9 | 2.9 |
| Shannon's diversity, H′ | 1.72 | 1.60 | 1.25 | 0.54 |

Total number of individuals and taxa, total biomass and Shannon's diversity index are given.

### 3.2. Litter decomposition

Mass loss of oak leaves was similar among sites until day 41 (23–31% AFDM lost). From this day forward, oak leaves at site S4 started to loose mass rapidly comparatively to the other sites. By day 63 mass loss varied from 30% at site S1 to 85% at site S4 (Fig. 3), being significantly higher at site S4 (ANCOVA, $p = 0.015$). This translated into slower decomposition rates at site S1 ($k = 0.0058$/d) and faster at site S4 ($k = 0.0273$/d) (Table 5). Mass loss of oak leaves across sites was positively related to the sporulation by aquatic hyphomycetes (linear regression, $p = 0.004$, $R^2 = 0.21$) and to the biomass of shredders (linear regression, $p = 0.007$, $R^2 = 0.99$). When calculating the ratio between the decomposition rates at sites S2, S3 and S4 and the decomposition rate at site S1 ($k_{impacted}$:$k_{reference}$), as proposed by Gessner and Chauvet (2002) as a measure of stream functional integrity, site S3 was considered as not compromised, site S2 was considered as having a compromised stream functioning and site S4 was considered as having a severely compromised stream functioning (Table 5).

### 3.3. Fungal sporulation

Fungal sporulation was ca. two times higher at sites S3 and S4 than at sites S1 and S2 until day 41 (two-way ANOVA, $p < 0.001$). After this date, sporulation rates at the downstream sites decreased sharply and no significant differences were found among sites when considering all sampling dates (two-way ANOVA, $p = 0.348$) (Fig. 4). Cumulative conidial production was higher at sites S3 and S4 than at sites S1 and S2 (ANCOVA, $p = 0.003$; Fig. 4).

### 3.4. Aquatic hyphomycete communities

Eighteen species of aquatic hyphomycetes sporulated on oak leaves in this study (Table 6) and although the number of species was related to sites (two-way ANOVA, $p = 0.047$) Tukey's test did not discriminate differences among sites. Aquatic hyphomycete communities associated with decomposing leaves were dominated by *Tetrachaetum elegans* at all sites, followed by *Tricladium chaetocladium* (S1), *Clavatospora longibrachiata* (S2) and *Alatospora acuminata* (S3 and S4) (Table 6). Five species had site dependent % contribution to total conidial production, with preferences for site S1 or S4 (two-way ANOVA; Table 6). Communities were more diverse (H′) and conidia more evenly distributed through species (J′) at sites S3 and S4 than at sites S1 and S2 (two-way ANOVA, $p < 0.001$; Table 6).

Sites S3 and S4 were the most different from site S1 (least impacted) when the aquatic hyphomycete communities

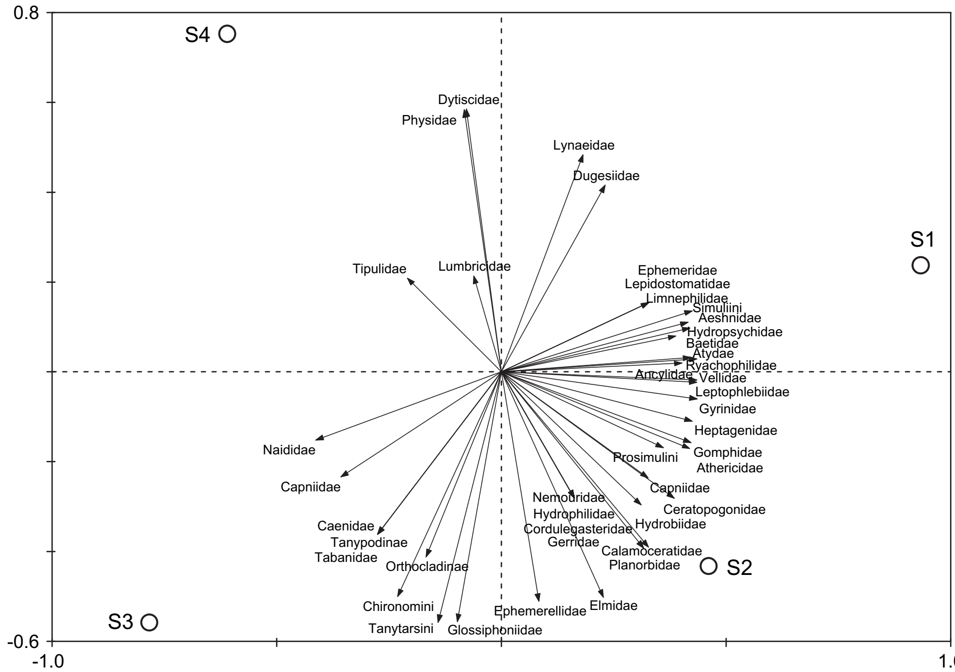


Fig. 2. Principal component analysis (PCA) for benthic macroinvertebrates at four sites along a habitat degradation gradient in Botão stream. PCA axis 1 explained 52% and PCA axis 2 explained 28% of variability among sites.

associated with oak leaves were analyzed by multivariate techniques (PRC; Fig. 5a); sites explained 55% of variability while time explained 31% (Monte Carlo permutation, $p = 0.002$). Species with the most positive scores (*C. longibrachiata*, 2.6) had a pattern similar to that described by the PRCs while species with the most negative scores (*T. elegans*, −3.3) had an opposite pattern (Fig. 5b).

## 4. Discussion

As expected from the location of the four sites assessed in Botão stream – upstream of the village with a native riparian corridor (S1), upstream of the village across a eucalyptus plantation (S2), within the village (S3) and below the village across agricultural fields (S4) – they differed with respect to water physico-chemical characteristics with downstream sites having higher SRP concentrations and higher SRP and $NO_3$ loads than upstream sites. They also differed in habitat quality; site S1 had a native riparian corridor and high substratum heterogeneity, site S2 crossed a eucalyptus plantation and sites S3 and S4 were severely altered by constraining and channelization (S3), removal of native riparian vegetation (S3 and S4) and agricultural fields (S4). These alterations were reflected in the IHF and QBR indices scores, which decreased from site S1 to S4, identifying a downstream degradation gradient. The effect of these differences among sites in the stream ecological integrity was evaluated by the assessment of both structural integrity, by means of a biotic index and benthic macroinvertebrates derived metrics, and functional integrity, by the use of litter decomposition rates. This was done by using protocols to conform to stream rapid bioassessment.

Measures of structural integrity discriminated sites S1 and S2 from sites S3 and S4. Indeed, pollution sensitive taxa (e.g. Ephemeroptera, Plecoptera and Trichoptera; Tachet et al., 2000) were higher at sites S1 and S2, which resulted

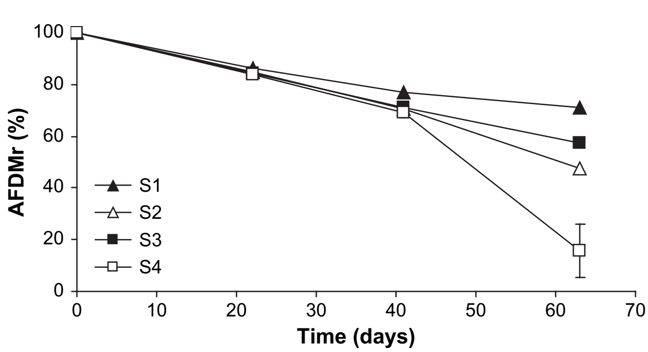


Fig. 3. Remaining mass (mean ± 1SE) of oak leaves incubated in coarse mesh bags at four sites along a habitat degradation gradient in Botão stream.

Table 5
Decomposition rates ($k$) of oak leaves incubated in coarse mesh bags along a habitat degradation gradient in Botão stream and ratio between the decomposition rates ($k$) at each impacted site (S2–S4) to decomposition rates at the reference site (S1) ($k_{imp}$:$k_{ref}$)

| Sites | $k$ (/d) | $R^2$ | $k_{imp}$:$k_{ref}$ [a] | Score |
|---|---|---|---|---|
| S1 | 0.0058 | 0.98 | | |
| S2 | 0.0113 | 0.78 | 1.69 | 1 |
| S3 | 0.0087 | 0.96 | 1.30 | 2 |
| S4 | 0.0273 | 0.66 | 4.07 | 0 |

[a] $0.75 < k_{imp}$:$k_{ref} < 1.33$, no clear evidence of impact, score 2; $1.33 < k_{imp}$:$k_{ref} < 2.0$, compromised river functioning, score 1; $k_{imp}$:$k_{ref} > 2.0$, severely compromised river functioning, score 0 (as defined by Gessner and Chauvet, 2002).

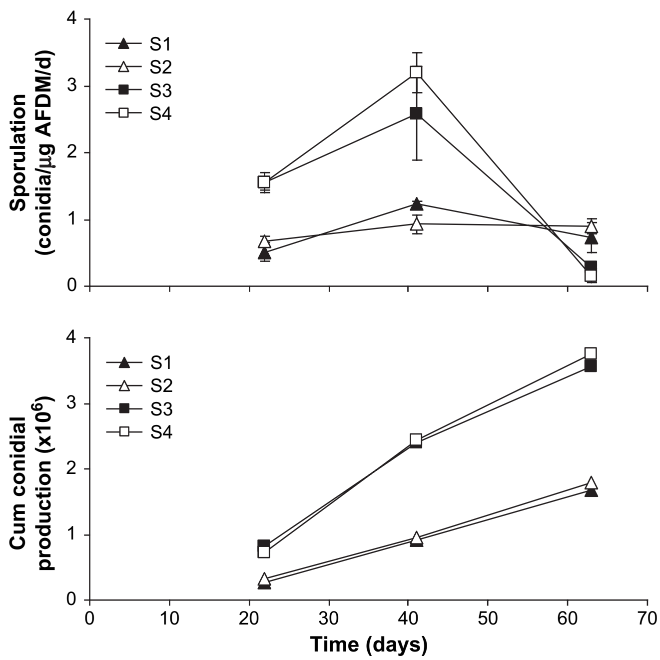


Fig. 4. Sporulation rates (mean ± 1SE) by aquatic hyphomycetes and cumulative conidial production in oak leaves incubated in coarse mesh bags at four sites along a habitat degradation gradient in Botão stream.

Table 6
Mean relative abundances (%, over all sampling dates) of aquatic hyphomycete conidia in oak leaves incubated in coarse mesh bags at four sites along a habitat degradation gradient in Botão stream

| | S1 | S2 | S3 | S4 |
|---|---|---|---|---|
| *Alatospora acuminata* sl*** | $4.8^{a}$ | $8.1^{a,b}$ | $14.5^{b,c}$ | $21.7^{c}$ |
| *Anguillospora* cf. *furtiva* | + | | | |
| *Anguillospora crassa* | 2.6 | 1.0 | 2.0 | 5.5 |
| *Anguillospora filiformis* | | + | 0.1 | |
| *Articulospora tetracladia* | 1.9 | 1.3 | 2.0 | 1.5 |
| *Clavariopsis aquatica*** | $1.2^{a,b}$ | $0.3^{b}$ | $5.2^{a}$ | $7.2^{a}$ |
| *Clavatospora longibrachiata** | $13.4^{a}$ | $15.1^{a}$ | $9.6^{a,b}$ | $10.8^{b}$ |
| *Lemonniera aquatica* | 0.3 | 0.6 | 1.2 | 0.3 |
| *Lunulospora curvula* | 5.7 | 3.8 | 5.1 | 6.9 |
| *Tetracladium marchalianum**** | $3.3^{a}$ | $4.6^{a,b}$ | $5.5^{b,c}$ | $10.5^{c}$ |
| *Tetrachaetum elegans* | 47.5 | 49.6 | 42.8 | 28.8 |
| *Tricladium chaetocladium** | $18.2^{a}$ | $14.9^{a,b}$ | $11.2^{a,b}$ | $6.3^{b}$ |
| *Tricladium splendens* | | 0.1 | | 0.2 |
| *Triscelophorus acuminatus* | 0.2 | 0.1 | 0.3 | 0.1 |
| *Triscelophorus monosporus* | 0.8 | 0.2 | 0.4 | |
| *Varicosporium* sp. | | 0.1 | | |
| Small irregular branched | | | | 0.2 |
| Unidentified tetraradiate | 0.1 | + | + | |
| Total no. of species | 14 | 16 | 14 | 13 |
| Pielou's equitability, J′ *** | $0.60^{a}$ (0.07) | $0.60^{a}$ (0.05) | $0.72^{b}$ (0.03) | $0.77^{b}$ (0.03) |
| Shannon's diversity, H′ *** | $1.24^{a}$ (0.18) | $1.24^{a}$ (0.11) | $1.63^{b}$ (0.06) | $1.67^{b}$ (0.06) |

Total number of species, mean Shannon's diversity and mean Pielou's evenness indices (1SE) are given.
+, mean relative abundances < 0.1%.
*$0.05 > p > 0.01$; **$0.01 > p > 0.001$; ***$p < 0.001$ (two-way ANOVA; sites with different letters are significantly different).

in higher IASPT scores, while pollution tolerant taxa (e.g. Diptera and Oligochaeta; Tachet et al., 2000) were higher at sites S3 and S4. The sensitivity of this type of benthic derived metrics to stream impairment has been widely reported (Maxted et al., 2002). The differential distribution of taxa across sites was translated into differences in the IBMWP scores that classified sites S1 and S2 as having very good water quality and sites S3 and S4 as having good and moderate water quality, respectively.

The score differences between the two groups of sites can be explained in two ways. First, they can be explained by differences in SRP concentration among sites. Although water chemical characteristics varied greatly between sampling dates, SRP concentrations at a given sampling date were always higher at sites S3 and S4 than at sites S1 and S2. Given the nature of biological data, which integrates environmental information across time, it is plausible that high SRP could be related with other unmeasured variables. For instance, high SRP may lead to high photosynthetic rates and eventual oxygen shortages during night. However, the precise cause–effect is not clear. In addition, IBMWP score variability can be explained by differences in habitat quality among sites; the BMWP′ biotic index (precursor of the IBMWP index) was shown to be highly sensitive to habitat heterogeneity (Ferreira et al., 2004). Indeed, sites S1 and S2 had abundant riparian vegetation which potentially provided food for shredders, many of which are classified as intolerant in the IBMWP table (e.g. Plecoptera, Trichoptera). Shredders were shown before to be sensitive to the presence of riparian vegetation (e.g. Wooster and DeBano, 2006) and Trichoptera species richness was shown to be related to tree species richness (Voelz and McArthur, 2000), which can partially explain the higher number of shredder taxa and individuals at the upstream sites when compared with sites S3 and S4. Moreover, Trichoptera are sensitive to substrate sizes (Velásquez and Miserendino, 2003; Feio et al., 2005) and coarse particles of substrate were scarce at sites S3 and S4. Overall, the high habitat quality at the upstream sites was expected to translate into high species richness at these sites, as different habitats are colonized by a particular species assemblage that presents faunal adaptations to that habitat (Pardo and Armitage, 1997). However, as high SRP concentrations and poor physical habitat occur together, a direct mechanism to explain their effect on macroinvertebrates cannot be deduced without further study.

The separation of sites upstream the village from sites within and downstream the village was also achieved when the entire benthic macroinvertebrate community was considered (PCA analysis), with sites distributed along axis 1 (52% variability explained) which was negatively correlated with SRP concentration in water and SRP and $NO_3$ loads. These results agree with those of Gulis et al. (2006) who reported a negative relationship between selected benthic metrics and dissolved nitrogen and phosphorus. On the other hand, a positive relationship between invertebrate density (Niyogi et al., 2003), biomass (Rosemond et al., 2002) and canonical analysis axis 1 coordinates (Pascoal et al., 2003) and phosphorus concentration in water was found before for the

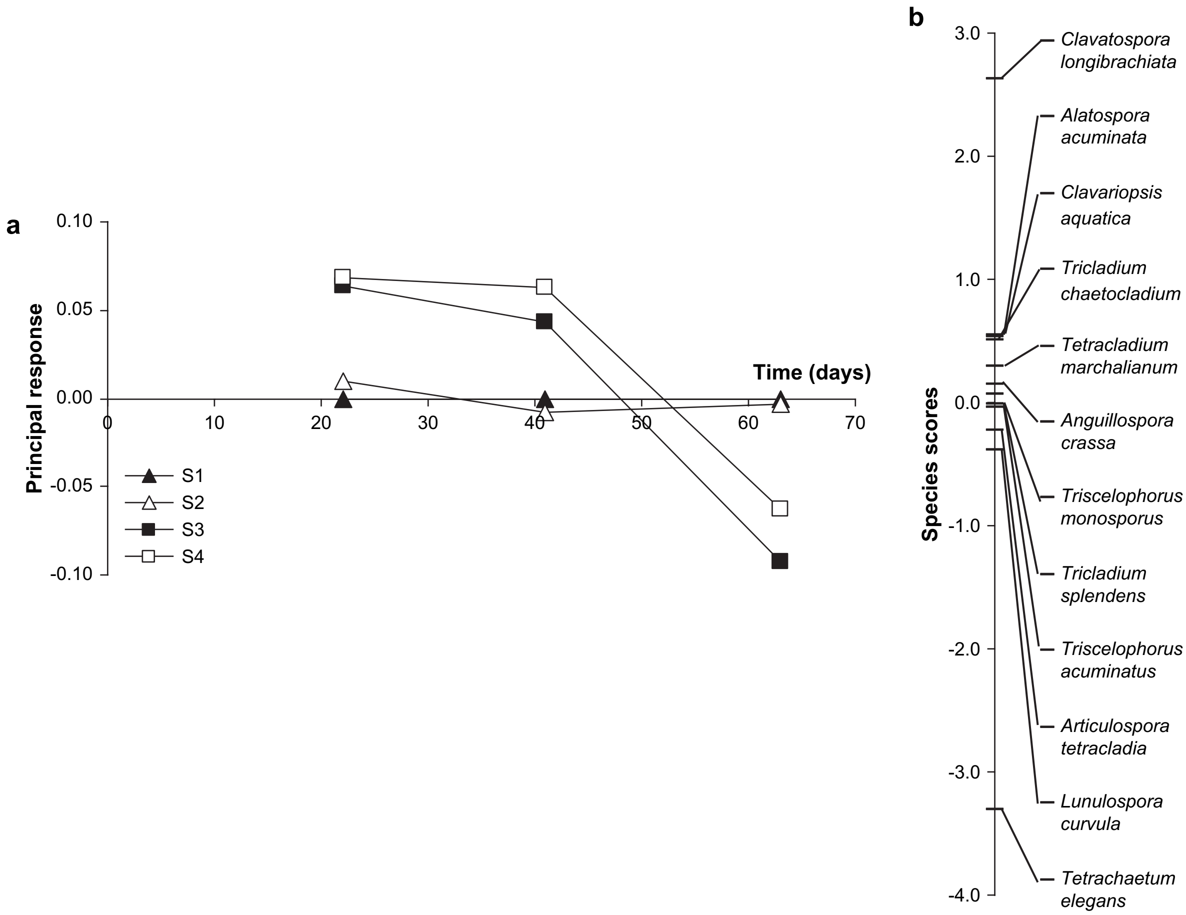


Fig. 5. (a) Principal response curves (PRC) for aquatic hyphomycetes communities in oak leaves incubated in coarse mesh bags at four sites along a habitat degradation gradient in Botão stream. Sites explained 55% and time explained 31% of variability among samples (Monte Carlo permutation, $p = 0.002$). (b) Most important aquatic hyphomycetes species defining differences among sites. Species with the most positive scores are positively correlated with the pattern described by the PRCs while the species with the most negative scores are negatively correlated with the same pattern.

same concentration range observed in this study. In our case, the higher SRP concentration was associated with lower habitat quality, which might explain differences between studies.

Decomposition rates of oak leaves across sites were similar to values reported for the same leaf species in Portugal at sites differing in nutrient concentration in water (Ferreira et al., 2006; Gulis et al., 2006). Mass loss of oak leaves was 1.7 times higher at site S2 (upstream the village) and 4 times higher at site S4 (below the village, across agricultural fields) than at site S1 (the most upstream, least impacted site), which according to Gessner and Chauvet (2002) indicates compromised and severely compromised ecosystem functioning, respectively. This partially agrees with differences in SRP concentration among sites. Elwood et al. (1981) found *Quercus rubra* leaves to decompose faster in P enriched than in control stream sections and Rosemond et al. (2002) found increasing decomposition rates of *Ficus insipida* leaves with increasing stream SRP concentration (up to ~25 μg/L) along a natural SRP gradient. High SRP concentrations probably stimulated litter mass loss through the stimulation of microbial activity; decomposition rates of oak leaves across sites were related to the sporulation by aquatic hyphomycetes, which are the most important microbial decomposers of submerged litter in temperate regions (Gulis and Suberkropp, 2003b; Pascoal and Cássio, 2004). However, site S3 was classified as not impacted ($k_{S3}/k_{S1} = 1.3$), although it had high SRP concentration (~16 μg/L), high nutrient loads and low quality of the riparian corridor (QBR = 20). Decomposition rates were also related to shredder biomass; the higher abundance of *Tipula* sp., a large crane fly larvae, at sites S4 (nine individuals) and S2 (four individuals) can help explaining the fast disappearance of leaves at these sites. In addition, the presence of macrophytes at site S3, which had the lowest percentage of shredders (1.9%) and the highest percentage of collectors (68%; at the other sites the percentage of collectors was 36–59%), may have contributed to the low mass loss of oak leaves at this site. The high percentage of collectors associated with macrophytes was observed on previous work (Velásquez and Miserendino, 2003).

At sites S3 and S4 aquatic hyphomycetes converted ca. 10% of the initial oak leaves AFDM into conidia while at sites S1 and S2 only ca. 5% were converted. Sporulation rates at the two upstream sites were similar to values reported for the same leaf species at low to moderate nutrient streams while sporulation at sites S3 and S4 was in the upper range reported for the same leaf species incubated at experimentally nitrogen enriched sites (Ferreira et al., 2006; Gulis et al., 2006). The higher fungal sporulation at sites S3 and S4 than at sites upstream of the village was probably due to higher SRP concentration at the downstream sites. Sporulation rates were indeed more discriminant among sites than decomposition rates. Several studies have shown that fungal sporulation is highly sensitive to the nutrient concentration in water (Sridhar and Bärlocher, 1997; Gulis and Suberkropp, 2003a), even more than decomposition rates (Sridhar and Bärlocher, 2000; Ferreira et al., 2006). On the other hand, a positive relationship between conidia production and the amount of benthic CPOM (Laitung et al., 2002) and riparian trees diversity (Wood-Eggenschwiler and Bärlocher, 1983) has been demonstrated. This was not observed in our study where higher sporulation was found at sites from where the native riparian vegetation was removed (S3) or substituted by the exotic *P. australis* (S4). Therefore, in our study fungal activity was more sensitive to water quality than benthic organic matter availability and diversity.

Similarly to the benthic macroinvertebrate communities, the aquatic hyphomycete communities discriminated between the two upstream sites (S1 and S2) and the sites within (S3) and below (S4) the village (PRC analysis). Although there were no differences in the number of species among sites, aquatic hyphomycete diversity was higher at sites S3 and S4. This discrimination between the two upstream and the two downstream sites was also done at the species level, with five species showing preference for site S1 or S4. Several species of aquatic hyphomycetes have been shown to have preferences for certain levels of dissolve nutrients (Ferreira et al., 2006). Although Suberkropp et al. (1988) and Graça (1994) found the aquatic hyphomycetes unreliable indicators of water quality as no differences in fungal community structure were observed on decomposing leaves incubated at sites with different nutrient concentrations, our data agree with those of Pascoal et al. (2005) who found organic enrichment to affect fungal diversity more than leaf decomposition rates.

## 5. Conclusions

The four study sites differed in SRP concentrations and physical characteristics, as they were located along a habitat degradation gradient described by nutrient enrichment, decreased riparian vegetation quality and decreased fluvial habitat quality. The benthic macroinvertebrate community was sensitive to these changes as it responded negatively to increases in SRP concentration, changes in the riparian vegetation from native to absent or exotic and to reduction in the habitat quality. Decomposition of oak leaves identified the most downstream site as having a severely compromised ecosystem functioning. However, the use of decomposition rates to assess stream functional integrity was limited by confounding factors (e.g. presence of macrophytes at site S3, see above). Aquatic hyphomycete reproductive activity was more affected by water quality than by habitat quality and more sensitive to water quality than decomposition rates. The aquatic hyphomycete communities associated with decomposing oak leaves also discriminated sites upstream from sites within and below the village due to a differential response of five species. The ecological evaluation of the four sites studied therefore indicates (a) site S4 as severely impaired in both structure and function, (b) site S3 as impaired in structure and (c) site S2 as impaired in function. If fungal reproductive activity (sporulation) is included in the evaluation, then site S3 is classified as impacted not only in structure but also in function. Although both the functional and the structural approaches used in this study gave the same results for the most impacted site (S4) they were complementary for moderately impacted sites (S2 and S3) and we therefore support the need for incorporating functional measures in evaluations of stream ecosystem integrity.

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